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ELECTROKINETIC STUDIES OF MARINE OVA [1]

II. Cumingia Tellinoides, Asterias Forbesii, Echinarachnius Parma, Nereis Limbata and Cerebratulus Lacteus

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In this study, an attempt was made to compare the cataphoretic potential of five types of marine eggs with that already described for sea-urchin eggs. The eggs chosen were those of the clam *Cumingia*, the starfish *Asterias*, the sand dollar *Echinarachnius*, the polychæte worm *Nereis*, and the nemertine *Cerebratulus*.

The method of measurement was the same as that described in the preceding paper (Dan, 1933). However, in this study the greater size of these eggs (except *Cumingia*) made necessary a slight modification in the procedure. The difficulty is that when, according to standard procedure, one tries to focus on an egg which is in the layer one-fifth of the distance across the chamber, the periphery of the egg comes so close to the wall that the latter exerts an influence on the movement of the egg. Strictly speaking, the egg is not free from this effect under any circumstances, but in case it lies sufficiently far from the wall, the effect becomes negligible. Therefore, I focussed to the middle layer of the chamber and studied the eggs in that layer. The effect of the electro-endosmotic current can be computed, as was described in the previous paper, from the graph obtained for this particular chamber. Another modification is the use of a lower magnification. *Nereis* eggs are very much heavier than the other types studied, and they therefore fall with a greater speed. As for *Asterias*, *Echinarachnius* and *Cerebratulus* eggs, even though their speed is not so great, their size is much larger than sea-urchin eggs and it is more convenient to observe them under a lower

[1] This paper and the preceding one of this series constitute a thesis presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

magnification. As a result, the accuracy of the data obtained in this way is not as great as that obtained by using a higher magnification, but it is, to be sure, good enough to determine the correct order of magnitude. When a lower magnification is used, the values obtained happen to be much smaller than those for *Arbacia* and *Cumingia* eggs, for which a higher magnification was adopted. This is, however, not an error due to the adoption of the lower magnification, for when *Arbacia* eggs with jelly are studied under similar conditions with low magnification, a charge of —33.0 millivolts is obtained, and this value differs only 1 millivolt in absolute magnitude from the value obtained previously.

Cumingia tellinoides

Unfertilized Eggs with Jelly.—Freshly collected animals, after being kept dry for a while, were transferred to Stender dishes, one animal in each dish. After several females had shed eggs, these eggs were put together and the potential was measured. Some of the results are given in Table I. The average value of 23 measurements is —34.1 millivolts.

TABLE I

Surface charge of unfertilized Cumingia eggs with jelly. Dielectric constant is taken as 80; viscosity is corrected to 25° C. (see text).

Potential gradient	Horizontal shift in 2.5 seconds	Values corrected for endosmosis and viscosity	Horizontal shift per second	Zeta potential
volt/cm.	*micra*		*micra*	*mv.*
	42.9	43.3	17.3	−34.0
	39.4	39.8	15.9	−31.1
	41.1	41.5	16.6	−32.6
6.7	47.0	47.4	19.0	−37.4
	41.6	42.0	16.8	−33.1
	41.6	42.0	16.8	−33.1
	45.8	46.2	18.5	−36.4
				Av. −34.0

In Table I, the dielectric constant of sea water is assumed to be 80, *i.e.*, that of distilled water. There are no measurements of the dielectric constant of sea water because of technical difficulties resulting from its high conductivity. As for the viscosity, the data for distilled water are used [2] and the cataphoretic speed is corrected to 25° C. to facilitate

[2] There is a measurement of viscosity of sea water by Krümmel and Ruppin (1905). If their figures are taken, the absolute magnitude of the zeta potential may become greater by 6–7 per cent. But, since the zeta potential itself can be only a relative measure in so far as our present knowledge goes, the data for distilled water are adopted in conformity with the previous work.

the comparison of these figures with those published in the preceding paper (Dan, 1933). The effect of temperature on the zeta potential itself is considered to be negligible within the range of temperature variation in this experiment (20°–24° C.) (Compare Burton, 1906).

Unfertilized Eggs without Jelly.—Eggs were secured in the same way as was described; then they were strained through bolting silk in order to remove the jelly. The result of 169 measurements gives the value of — 28.8 millivolts, the standard error being ± 0.46.

Asterias forbesii

Unfertilized Eggs with Jelly.—Eggs were obtained by cutting ovaries out of the body of a female and allowing mature eggs to stream out freely into the sea water. After fifteen or twenty minutes, the measurement was started. Eighty-two such measurements give an average value of — 19.0 ± 1.04 millivolts.

It may be noticed that the standard error is much greater here due to the adoption of a lower magnification. However, this is due mostly to the fluctuation involved in the process of free-hand tracing, as was discussed in the previous paper. This is shown by the fact that when eggs are cytolyzed and their speed of fall is thereby reduced, the measurement becomes relatively more accurate and a lower figure for the standard error can again be obtained, as will be seen later.

Unfertilized Eggs without Jelly.—Eggs, secured by the method which was described in the preceding section, were shaken vigorously so that the jelly would be removed. After shaking, samples were taken and were examined in Chinese ink suspension to determine whether or not the removal of the jelly was complete. Ninety-nine measurements were made, giving — 19.9 ± 1.01 millivolts as the average value. This figure is practically identical with that of eggs with jelly. In *Arbacia* and *Cumingia*, as well as in *Echinarachnius* (see below), the jelly shows a much higher absolute cataphoretic potential than the eggs. In so far as the data accumulated thus far are concerned, the *Asterias* egg is the only one in which the jelly has the same charge as the egg surface.

Eggs Killed by Heat.—There have been several studies concerning the effect of death upon the surface charge of cells. As early as 1906, Cernovodeanu and Henri discovered that bacteria retained their original charge materially unchanged, even after death of the cells by heat. Russ (1909) observed a similar fact with tubercle bacilli in urine. More recently Winslow et al. (1923) confirmed the earlier finding by using a more elaborate technique. Beside these facts, Abramson (1931) reported that "ghosts" of hæmolysed blood cells travelled with the identi-

cal cataphoretic velocity as that of intact cells. These observations are of great interest, but since they are concerned with rather simple and more or less quiescent materials such as bacteria or blood cells, it is very important to discover how more active cells would behave under similar circumstances.

Abramson (1928) made an observation in this direction. In his study of polymorphonuclear leucocytes, he compared the potential of active cells with that of degenerating cells. The latter were spherical in shape and non-amœboid. However, no difference was found between the speed of active and degenerating cells. He also pointed out that if human white cells were kept on ice for two days, there was no change

TABLE II

Surface charge of Asterias eggs (without jelly) killed by subjecting them to 40° C. for 5 minutes. Dielectric constant is taken as 80; viscosity is corrected to 25° C.

Potential gradient	Horizontal shift in 2.5 seconds	Values corrected for endosmosis and viscosity	Horizontal shift per second	Zeta potential
volt/cm.	*micra*		*micra*	*mv.*
	31.5	25.9	10.3	−20.2
	31.5	25.9	10.3	−20.2
	25.7	20.1	8.0	−15.7
	31.5	25.9	10.3	−20.2
6.7	36.2	29.4	11.8	−23.0
	24.5	18.9	7.6	−14.9
	29.1	23.5	9.4	−18.4
	33.9	28.3	11.3	−22.1
	31.5	25.9	10.3	−20.2
				Av. −19.4

in the cataphoretic speed. With these facts in mind, the following experiment was tried on *Asterias* eggs.

Eggs were obtained in the same manner as before and the jelly was shaken off. Then the eggs were subjected to 40° C. for 5 minutes. At the end of this period, all the eggs were completely cytolyzed, and the average diameter, which was 146.8 μ before the treatment, now measured 174.6 μ. The cytolysis was so complete that there was no chance for any eggs to survive the treatment. Some of the results are given in Table II. The final figure from 85 measurements is -19.0 ± 0.49 millivolts. It is surprising that the value for eggs killed by heat is identical with that of normal living eggs, in spite of the fact that the diameter and even the appearance of the cell has been so much changed by cytolysis.

Echinarachnius parma

Eggs were removed from females by cutting the tests open and putting ripe ovaries in an ample amount of sea water. Eggs were washed carefully. From 23 measurements, the average value for the potential of eggs with jelly is found to be —31.6 millivolts. Occasionally a piece of jelly which had been detached from an egg cell was found. In such a case, even though the jelly itself is invisible because of its transparency, its presence can be detected by the pigment granules which are scattered through it. If a piece of this sort was selected and its cataphoretic speed measured, it was found to move with about the same speed as intact jelly still attached to the surface of the cell.

In this form it is rather hard to get eggs free from jelly, because of the delicacy of the egg cells, so that it was never certain whether or not the removal of jelly was complete. However, eggs which were, so far as could be determined, without jelly, moved about two-thirds as fast as those with jelly. This fact may indicate that the potential of the naked surfaces of eggs falls somewhere around —20.0 millivolts. In one experiment tissue cells were involved in the egg suspension, and these tissue cells showed a speed of the same order of magnitude as egg cells without jelly.

Nereis limbata (Unfertilized Eggs)

The material was collected in the evening and was sometimes used immediately. Often, however, it was experimented upon early the following morning. The body of a female was cut open, the eggs were washed carefully, and they were then quickly brought into the chamber. Because of the great speed of fall of these eggs, the measurements are not as accurate as the preceding ones. The mean value of 76 measurements is —9.7 millivolts.

Cerebratulus lacteus (Unfertilized Eggs)

Eggs were secured by cutting open the body of a female. Some eggs were irregular in shape immediately after being taken out of the body, but later they became more spherical. The experiments were performed only after this state was reached.

As is well known, the *Cerebratulus* egg is enclosed in a huge chorion. This chorionic membrane has a certain degree of rigidity, but it is not difficult to remove it if desired. The large space within the membrane seems to be filled with some sort of a viscous organic fluid, for when seen from the side, the egg cell is always suspended, whereas if it is

taken out into the sea water, it sinks very rapidly. Therefore in this case we must take account of the fact that the system we are dealing with is a composite one, consisting of three component parts: chorionic membrane, chorionic fluid, and egg cell. This is a very important point to keep in mind.

Surface Charge of the Chorion.—First, a chorion containing an egg was put into the cataphoresis chamber and its behavior in the electric current was observed. The chorion of *Cerebratulus* has an ovoidal form. Its long axis measures about 300 μ and the short axis about 200 μ. Therefore, taking 250 μ as the average diameter, for the purpose of a rough calculation, the strength of the endosmotic current which is originated by the wall of the cataphoresis chamber and is acting on the chorion is calculated. If this is done, since the observed speed is the addition of the true cataphoretic speed and the shift caused by the electroendosmotic flow, the former can easily be known. As a result of this calculation, it was found that the speed of migration of the chorion toward the anode far exceeded that which might be attributed to the electroendosmotic current acting on the chorion. Thus it is certain that the surface of the chorion is negatively charged.

Migration of the Egg Cell within the Chorion.—During the course of various experiments, chorions were sometimes found which, for some reason, were attached to the wall of the chamber. In this case, the chorion was incapable of free movement, and the behavior of the egg cell suspended in the chorionic fluid could be studied. The striking thing which was found in these cases was the migration of the egg cell toward the cathode (it is thus apparently positive). The speed of this cathodic migration was very small and, so far, no reliable figure has been obtained. After the egg reached one end of the chorion, it became somewhat flattened against the membrane, while the membrane itself was made to bulge out under the pressure of the egg. If the current was reversed, the egg regained its spherical form, changed its direction of migration, and travelled across the chorionic space very slowly. When it came to the other end, the same flattening occurred. This could be repeated many times with the same egg by reversing the current. The accompanying camera lucida drawing (Fig. 1) illustrates the phenomenon above described. This sketch, however, does not represent the highest degree of flattening, for the breaking of the current to permit drawing allowed the egg to resume its spherical form.

Movement of the Egg Cell in Sea Water.—In other experiments, the chorion was removed by straining the egg through bolting silk. The potential on the naked surface of the egg cell was measured in the same way as was done in the case of *Asterias* and other eggs. The mean

value of 16 readings was found to be — 1.4 millivolts. Unfortunately 16 measurements is a very small number. *Cerebratulus* material is very difficult to obtain at Woods Hole, and only one worm was available during the entire summer of 1932 and none in 1933. As a result of the small number of determinations and the relatively high standard error, the value arrived at is not sufficiently reliable to establish the negativity of the potential. A greater number of determinations might very well give a small positive value, which would explain the slow cathodic migration of the eggs mentioned above. At any rate, for the present, it can at least be said with certainty that the charge on *Cerebratulus* eggs is in the vicinity of zero.

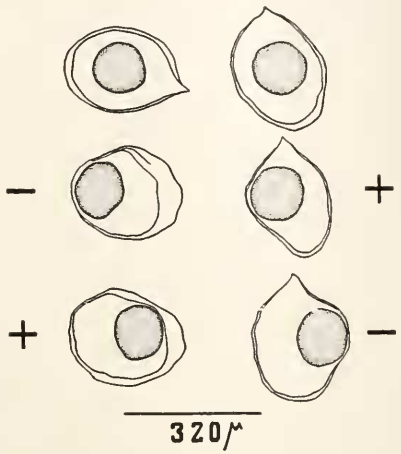

FIG. 1. Eggs of *Cerebratulus lacteus* within chorions. Each column represents the behavior of a single egg before and after the application of the electric current. Note the cathodic migration of the cells within their chorions.

In spite of the drawback in the measurement above stated, this egg indicates many interesting features. The first point to be noted is the peculiar fact that *Cerebratulus* eggs show so small a potential in such an alkaline medium as sea water (pH 8.2). Of course, there are several kinds of bacteria known to have a very low potential (in absolute magnitude) through a wide range of hydrogen ion concentration (Mudd and Joffe, 1933). Also there are several observations of the acquisition of zero or even a positive potential by bacteria in high pH values. The paper of Winslow et al. quoted above (1923) serves as one example of this phenomenon. Moreover, Vlès and Nouel (1922) and later Vlès (1924), in their experiments on agglutination of sea-urchin eggs at various pH values, found that there was a secondary agglutination point

at an extremely high pH region. This might indicate, though indirectly, that sea-urchin eggs (*Paracentrotus lividus* Lk.) have a secondary isoelectric point in an extremely alkaline medium. At present, however, experimental data are too scanty to warrant speculation concerning the *Cerebratulus* egg. It is hoped that the investigation can be extended in this direction in the near future.

The second point of interest is the migration of these eggs within the chorion. This naturally leads to the question whether or not other eggs which are surrounded by transparent jelly behave in a similar way in an electric field. Therefore a qualitative observation was undertaken by using the eggs of *Echinarachnius, Asterias,* and *Arbacia.* The experiment consisted of sending an electric current through an India ink suspension containing eggs under cover slips. As soon as the circuit was made, the existence of a strong electroendosmotic current was revealed by the fact that all the ink particles on the cathodal side of the jelly were completely washed away from its vicinity. This is due to a current of water flowing through the jelly from the anodal side to the cathodal side, as is to be expected from the negative charge on the jelly noted above. However, in spite of this striking change in the surrounding medium, no shift was observed in the relative positions of the jelly and the egg cell. This is probably due to the difference in the physical nature of the chorionic fluid of *Cerebratulus* and that of the jelly layers of other eggs. On the other hand, Freundlich and Abramson (1927) discovered that red-blood cells migrate as fast cataphoretically in 1 per cent gelatine gel as in a sol of the same concentration. However, until the exact physical nature of the jelly is known, no conclusion can be drawn.

As for the flattening of the cell against the chorionic wall, Mazia (1933) reported a strikingly similar behavior in frog eggs (with jelly), and he also found that frog eggs migrated toward the cathode even after the jelly was removed by KCN. Another similar phenomenon was observed long ago by Carlgren (1900) in the parthenogonidia of *Volvox,* although the direction of the migration was opposite, namely toward the anode. Since I have not had the opportunity to study this material, I venture no opinion concerning it. At any rate, it is remarkable that a cell which is surrounded by a certain structure can migrate and flatten itself against the wall of its containing envelope.

Bungenberg de Jong (1932) has studied a somewhat similar phenomenon in non-living systems, and it is instructive to compare the analogous behavior of living and non-living systems.

Here I acknowledge my great indebtedness to Dr. L. V. Heilbrunn under whose direction this work was completed.

Summary

1. By means of the method described in an earlier paper and a slight modification of it, the surface charge of five kinds of marine ova was measured.

2. *Cumingia* eggs with jelly show a charge of — 34.1 millivolts (23 measurements); eggs without jelly show a charge of — 28.8 ± 0.46 millivolts.

3. *Asterias* eggs with jelly have a charge of — 19.0 ± 1.04 millivolts; those without jelly — 19.9 ± 1.01 millivolts; and eggs without jelly which were killed by heat have a charge of — 19.0 ± 0.49 millivolts.

4. *Echinarachnius* eggs with jelly have a charge of — 31.6 millivolts (23 measurements), and eggs without jelly seem to have a charge of about — 20.0 millivolts.

5. *Nereis* eggs, according to less accurate data, show a charge of about — 10.0 millivolts.

6. In the case of the *Cerebratulus* egg, the negative charge of the chorionic membrane was first ascertained; next the cathodic migration of the egg cell within the chorion was observed; and finally the surface charge of the cell in sea water was demonstrated to be in the vicinity of zero.

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